(and/or its cellular source) will likely be required in order (1) to prevent recurrence of complement-mediated injury and (2) to prevent graft pathology induced by complement-independent mechanisms initiated by the alloantibody. Newer agents targeting B cells and plasma cells, including proteosome inhibitors that are currently being tested in transplantation,[4] may prove to be more efficacious than currently available reagents for this purpose.

The report by Tillou *et al.*[6] represents a small but important step toward developing an effective therapeutic strategy for antibody-mediated transplant rejection. The findings support further development and testing of the safety and efficacy of complement inhibition as part of a comprehensive strategy to treat this disease in humans.

**DISCLOSURE**
The author declared no competing interests.

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# The nanopeptide hormone vasopressin is a new player in the modulation of renal $Na^+$–$Cl^-$ cotransporter activity

Gerardo Gamba[1,2,3]

**Vasopressin is a modulator of salt and water reabsorption, with known effects in the thick ascending limb and the collecting duct. Pedersen *et al.* present evidence that vasopressin administration increases the phosphorylation of the apical thiazide-sensitive $Na^+$–$Cl^-$ cotransporter in the distal convoluted tubule. These effects appear to be independent of the renin–angiotensin system and to be mediated by the intracellular kinase SPAK. These observations expand the vasopressin-sensitive region of the nephron.**

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Regulation of salt reabsorption in the distal nephron, that is, the tubular segments located beyond the macula densa, plays a key role in defining the final concentration of salt in urine and thus, on a long-term basis, helps define blood pressure levels. To date, all monogenic diseases featuring arterial hypertension or hypotension in which the diseased gene has been discovered are due to mutations in genes that encode proteins known to be directly involved in salt reabsorption in the distal nephron. One of these genes is *SLC12A3*, encoding the thiazide-sensitive $Na^+$–$Cl^-$ cotransporter (NCC), which is heavily expressed in the apical membrane of the distal convoluted tubule (DCT). The activity of NCC is important for salt reabsorption and also for potassium secretion, because the salt reabsorption rate in the DCT defines sodium delivery to the collecting duct, which is necessary for the sodium/potassium exchange between the epithelial sodium channel, ENaC, and the apical potassium channels ROMK and BK in order to promote potassium secretion. The importance of NCC in blood pressure regulation has been clearly demonstrated over the years, as inhibition of NCC with thiazides reduces blood pressure in many hypertensive patients. Additionally, inactivating mutations of the *SLC12A3* gene are the cause of Gitelman's disease, an inherited syndrome featuring arterial hypotension and hypokalemia. On the other hand, loss of proper regulation of NCC by the mutant with-no-lysine serine/threonine kinases (WNKs) WNK1 and WNK4 seems to be an important mechanism for the development of the arterial hypertension and hyperkalemia seen in pseudohypoaldosteronism type II.[1] Thus, modulation of NCC activity is a growing field of study, since as we learn about how this cotransporter is regulated, we increase the possibilities for understanding complex

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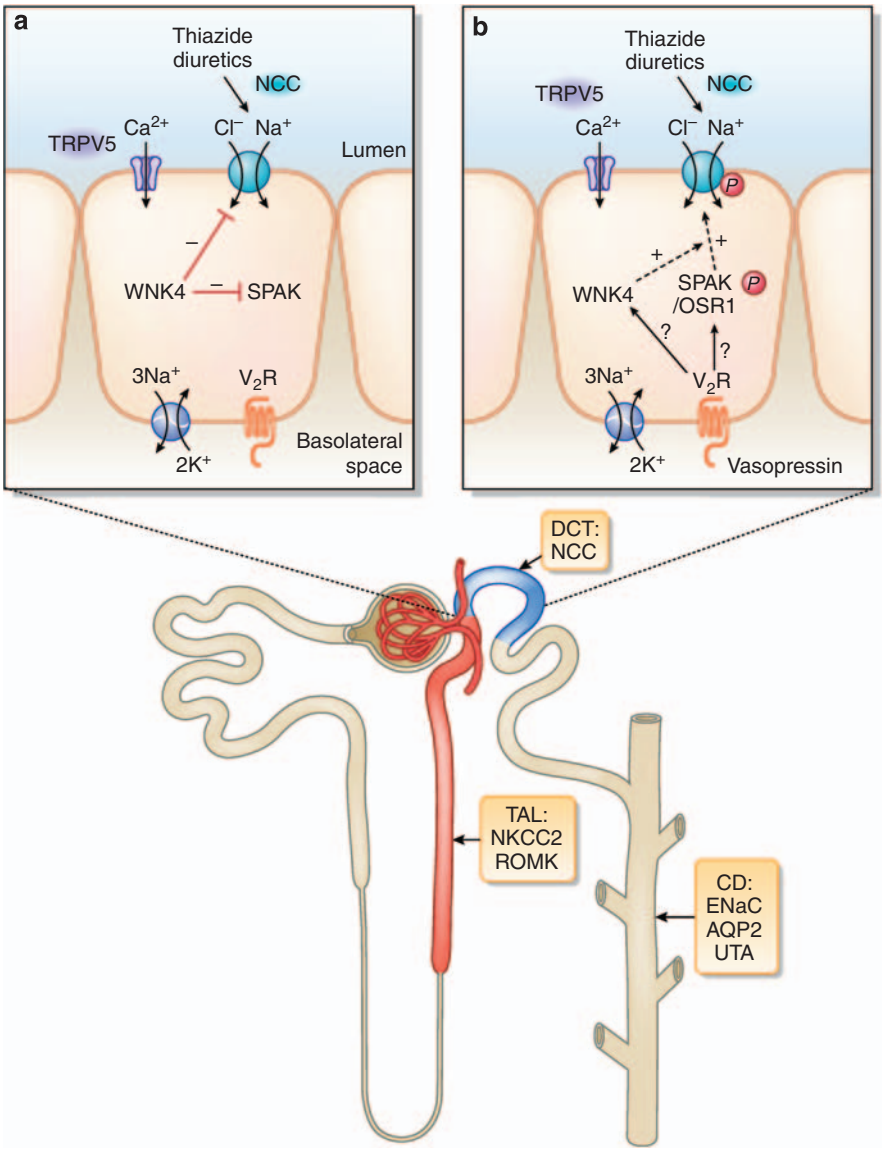


**Figure 1 | Known vasopressin actions in the nephron.** Vasopressin is well known for its activation properties in renal tubules via its interaction with the $V_2$-type receptor, particularly in the thick ascending limb of Henle (TAL) and collecting duct (CD). Pedersen *et al.*[8] show that vasopressin is also an activator of the $Na^+$–$Cl^-$ cotransporter (NCC) in the distal convoluted tubule (DCT). (**a**) The DCT in the absence of vasopressin. (**b**) The DCT in the presence of vasopressin.

diseases such as essential hypertension and developing newer antihypertensive drugs and strategies.

Major advances have been produced in the past decade regarding modulation of NCC. The activity of this cotransporter, as well as the closely related members of its family, the $Na^+$–$K^+$–$2Cl^-$ cotransporters NKCC1 and NKCC2, is associated with phosphorylation of certain conserved threonine residues in the amino-terminal domain.[2–4] Thus, antibodies recognizing these threonines when phosphorylated (phosphoantibodies) can now be used as a tool to indirectly assess activity of these cotransporters in several *in vitro* and *in vivo* models. In rat NCC, threonine-53 (T53), T58, and serine-71 (S71) have been recognized as the most important ones, corresponding to T55, T60, and S73 in humans.[2,5] So far, the kinases that seem to be responsible for the phosphorylation of these sites are the STE20-related kinases known as STE20/SPS1-related proline-alanine-rich kinase (SPAK) and oxidative stress-responsive kinase-1 (OSR1). Consistent with this, a recent report shows that elimination of SPAK's activity in mice produces a Gitelman-like syndrome, with arterial hypotension and hypokalemia,[6] due to a decreased expression and phosphorylation of NCC. SPAK and OSR1 lie downstream of the WNKs,[4,7] kinases that cause pseudohypoaldosteronism type II, which presents with arterial hypertension. Thus, the current paradigm is that WNKs (WNK3, WNK4) interact with and phosphorylate SPAK, which in turn phosphorylates NCC, increasing its activity. It has not been clearly established whether NCC (or NKCC1/2) phosphorylation at amino-terminal threonine residues increases the trafficking of the cotransporter to the plasma membrane or the activity of the transporters already in the membrane.

Most of the hormones that modulate blood pressure levels do so by affecting vascular smooth muscle contraction and/or urinary salt and water metabolism. Examples are the renin–angiotensin–aldosterone system, the sympathetic nervous system, and atrial natriuretic peptide. Vasopressin is another hormone that has clear effects on both blood vessels and renal tubules. While this peptide produces vasoconstriction by activating Gαq-coupled $V_1$ membrane receptors in blood vessels, it also increases salt reabsorption in the thick ascending limb and water in the collecting duct, through activation of its Gαs-coupled $V_2$ membrane receptors (Figure 1). The main consequence of these renal actions, however, is the urine concentration ability, rather than modulation of arterial blood pressure. Thus, an effect of vasopressin on the DCT to increase salt reabsorption has been suggested for years and is supported by some physiological studies, but elucidation of such effects has been limited because of the absence of reliable culture cells from the DCT.

Pedersen *et al.*[8] (this issue) now present compelling evidence that vasopressin positively modulates NCC activity. The authors took advantage of the fact that NCC phosphorylation at the amino-terminal domain threonines

has become a useful molecular tool to indirectly assess NCC activity. In this study, phosphoantibodies directed against phosphorylated NCC at T53 and T58 were raised and characterized. Using this tool, the authors made a series of interesting observations. First, acute or chronic treatment of arginine vasopressin (AVP)-deficient Brattleboro rats with the $V_2$ receptor-selective analog 1-desamino-8-D-arginine-AVP (dDAVP) significantly increased phosphorylation of NCC at T53 and T58. This observation strongly suggests that vasopressin modulates NCC activity. Second, by means of immunohistochemistry and immunogold electron microscopy of Brattleboro kidney sections, the authors observed that dDAVP induces phosphorylation of NCC particles that are already in the membrane, but not of those in the submembranal space. In other words, they propose that dDAVP does not alter the subcellular distribution of NCC, implying that phosphorylation of NCC is associated with increased activity of transporters that are already in the apical membrane, rather than stimulating trafficking of NCC vesicles. Third, the authors show that an effect of dDAVP is present even in rats in which angiotensin II actions have been maximally reduced by a high-salt diet and treatment with the angiotensin type 1 receptor blocker candesartan, reducing the possibility that vasopressin effects are due to activation of the renin–angiotensin system. They also show a transient increase of intracellular calcium induced by dDAVP on isolated distal tubules, suggesting a direct effect of vasopressin in the DCT that requires no intermediaries. The effect was much more intense in the late DCT than in the early DCT. Finally, by using specific antibodies to recognize the total and phosphorylated SPAK and OSR1 kinase on residues T240/T185 and S380/S325, the authors observed that dDAVP administration was associated with a slight but significant increase in SPAK and OSR1 phosphorylation, suggesting that SPAK and OSR1 are involved in the vasopressin-induced phosphorylation of NCC.

The results presented by Pedersen *et al.*[8] represent an advance in our knowledge of NCC modulation by a hormone produced outside the kidney, and also in our understanding of the diverse effects vasopressin has in different regions of the nephron. With this information we can now consider vasopressin as a hormone that has a positive effect on salt and water reabsorption all the way from the thick ascending limb of Henle to the collecting duct (Figure 1). Interestingly, a recent publication by another group[9] using a similar model of Brattleboro rats treated with dDAVP presented evidence that vasopressin induces activation of NCC by increasing phosphorylation of T53 and S71. S71 is another phosphoacceptor site that has been shown to be associated with increased activity of NCC when phosphorylated.[2] Although both reports agree on the major message that vasopressin activates NCC, intriguingly, there are some observations that are completely opposite from one study to the other. Pedersen *et al.*[8] suggest that vasopressin induces phosphorylation of NCC that is already located in the apical plasma. This observation is supported by a study using *Xenopus laevis* oocytes[2] in which induction or prevention of NCC phosphorylation at T53, T58, or S71 was associated with increased or decreased activity of NCC, respectively, without affecting its amount in the plasma membrane. In contrast, Mutig *et al.*[9] suggest that vasopressin modulates the trafficking of NCC particles to the apical membrane. This is supported by the previous observation that vasopressin increases trafficking of NKCC2 to the plasma membrane by inducing phosphorylation of the same residues.[10] There is a slight difference in the route and dose of dDAVP administration between the two studies, but it is unlikely that this could be the explanation for the opposite results. Another interesting difference is the location of the vasopressin effect on the DCT. According to Pedersen *et al.*,[8] the effect of vasopressin was observed in both DCT1 and DCT2 and was more intensive in DCT2, whereas Mutig *et al.*[9] present evidence that the vasopressin effect occurs only in DCT1. More detailed analysis of this issue is required, but, as was mentioned earlier, both studies agree on the major message that vasopressin modulates NCC activity.

Finally, it is interesting that the observations of Pedersen *et al.*[8] suggest that vasopressin activates NCC by phosphorylating the cotransporter through the SPAK/OSR1 kinases. Since it is known that SPAK and OSR1 lie downstream of the WNK kinases,[4,5,7] it is possible that vasopressin's activation of its Gαs-coupled receptor modulates the activity and phosphorylation of the WNKs.

## DISCLOSURE

The author declared no competing interests.

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